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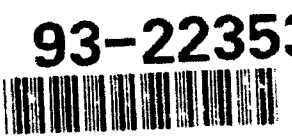
Long-Term Effects of Dredging Operations Program

Chronic Sublethal Effects of San Francisco Bay Sediments on *Nereis (Neanthes) arenaceodentata*; Effect of Food Ration on Sediment Toxicity

by *David W. Moore, Thomas M. Dillon*
Environmental Laboratory



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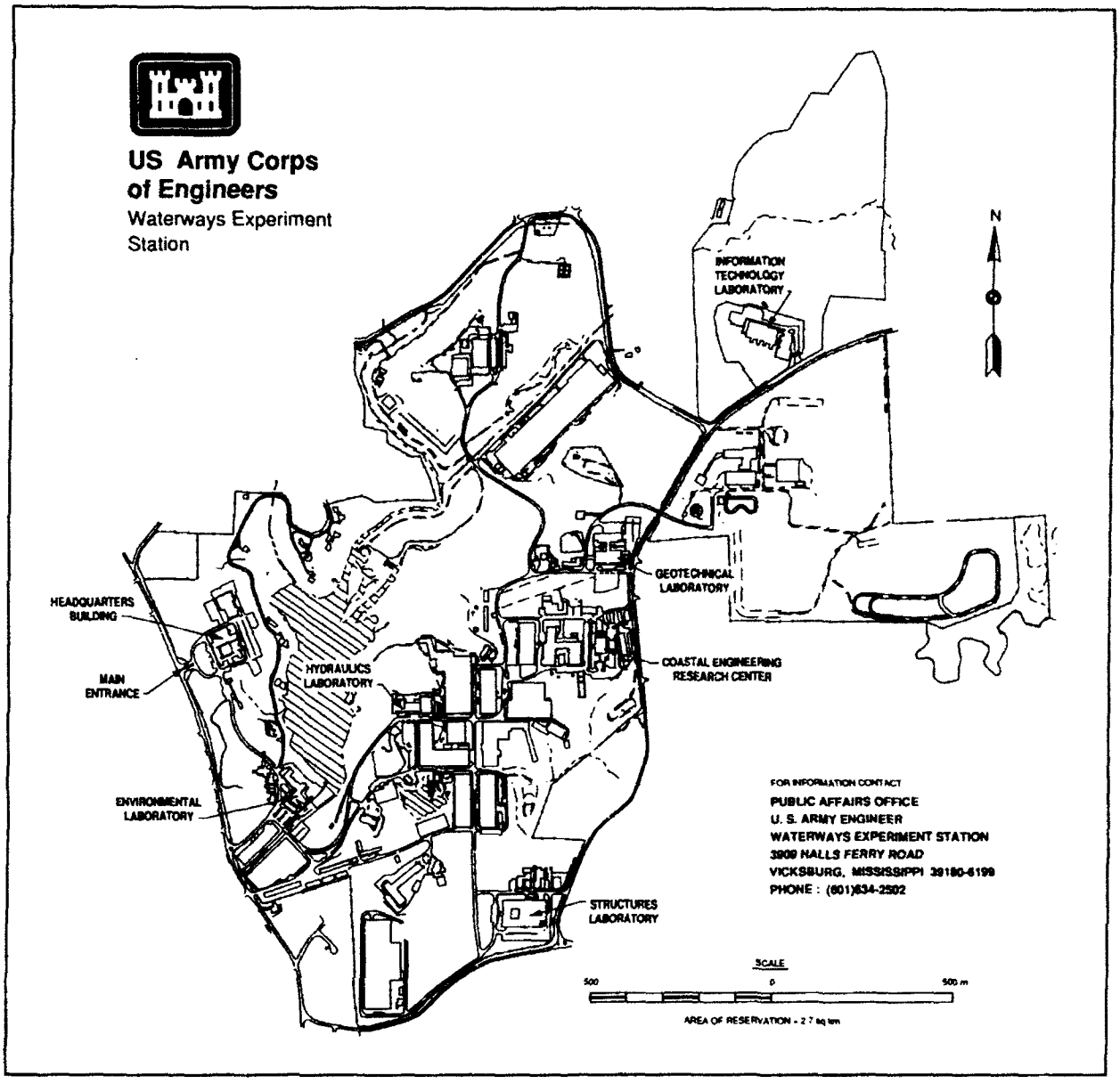

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Preface

The work reported herein was conducted by the U.S. Army Engineer Waterways Experiment Station (WES) for Headquarters, U.S. Army Corps of Engineers (HQUSACE), and the U.S. Army Engineer District (USAED), San Francisco. Financial support was provided by the USAED, San Francisco, through an Intra-Army Order for Reimbursable Services. Additional funding was provided by HQUSACE through the Long-Term Effects of Dredging Operations (LEDO) Program, Work Unit 374-9, "Chronic Sublethal Effects." The LEDO Program is managed through the Environmental Effects of Dredging Programs, Dr. R. M. Engler, Manager.

This report was prepared by Drs. David W. Moore and Thomas M. Dillon of the Environmental Processes and Effects Division (EPED), Environmental Laboratory (EL), WES. The authors gratefully acknowledge the support provided by personnel at the USAED, San Francisco, Mr. Thomas Chase, Mr. Kerry Guy, Ms. Sandra Lemlich (currently with the Coastal Engineering Research Center, WES), Mr. Duke Roberts, Mr. Thomas Wakeman, and Mr. Brian Walls (currently with Battelle, Duxbury, MA).

The work was performed under the general supervision of Dr. Bobby L. Folsom, Jr., Chief, Fate and Effects Branch, EPED; Mr. Donald L. Robey, Chief, EPED; and Dr. John Harrison, Chief, EL.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

Background

San Francisco Bay is a highly altered estuary. Two of the major reasons for this condition are the diversion of freshwater inflow from the Sacramento-San Joaquin River systems and the loss of wetlands.

By 1980, nearly 60 percent of the historic freshwater inflow to San Francisco Bay estuary had been diverted, mostly for agricultural irrigation. This reduction is projected to increase an additional 10 percent by the year 2000. About 95 percent of all freshwater/estuarine marshlands have been lost to land reclamation since 1850. It is not surprising, therefore, that the estuary has experienced a general decline in health and viability. One of the more noticeable symptoms of this decline has been the gradual loss of biological resources such as the striped bass and Pacific herring fisheries (Nichols et al. 1986).

An increase in the input of environmental contaminants has accompanied the physical alterations to San Francisco Bay. Major pollutant sources include metals associated with mining tailings located in Sacramento-San Joaquin River drainage basins. Additionally, over 50 waste treatment plants and about 200 industries are permitted to discharge directly into the Bay (Luoma and Phillips 1988). Environmental contaminants discharged into aqueous systems tend to associate with particulate material in the water column and with bedded sediments. Periodically, these bedded sediments must be removed to maintain navigable waterways. There is concern that the relocation of these dredged materials may be having unacceptable adverse impacts on aquatic biota within San Francisco Bay.

A large amount of sediment is dredged each year in San Francisco Bay. Approximately 5.5 million cubic meters (mcm) of sediment from Federal projects and permit actions are relocated annually. This value approximates the estimated average annual sediment inflow from natural sources of 6 to 8 mcm U.S. Army Corps of Engineers (USACE 1979). It has been estimated that 3.0 to 4.0 mcm of material leaves the Bay annually, while Central and North Bays experience a combined net accumulation of 4.2 mcm (USACE 1979). South Bay shows a net loss of nearly 0.8 mcm per year (Krone 1979). Despite

these large numbers, the greatest yearly source of suspended sediment in San Francisco Bay is the resuspension of existing bottom material. Approximately 120 to 130 mcm of sediment are resuspended each year by wind waves and currents (USACE 1979). The effect of these resuspended sediments on fish and aquatic invertebrates is unknown.

To examine whether San Francisco Bay dredged material was causing adverse biological effects, the Planning and Engineering Division of the U.S. Army Engineer District, San Francisco, contracted with the Environmental Laboratory of the U.S. Army Engineer Waterways Experiment Station (WES) to develop and conduct a series of chronic sublethal sediment bioassays using material from selected sites within the Bay.

Regulatory History of Dredged Material Management in San Francisco Bay

To help define what is known regarding the potential toxicity of San Francisco Bay sediments, it is useful to first examine how dredged material has been regulated in the past. Important milestones in that process are shown in Table 1. It was recognized very early that San Francisco Bay is a physically dynamic system and that most dredged material disposal sites were dispersive. Consequently, initial management concerns were mostly operational. That is, efforts were directed towards optimizing dredging and disposal operations to minimize transportation costs and redredging.

Passage of the National Environmental Policy Act in 1970 outlined the Federal Government's policy toward the environment and signaled an increasing desire for environmental protection in this country. That same year, the San Francisco District initiated the Dredge Disposal Study (DDS) (USACE 1977). The DDS was a multifaceted interdisciplinary study designed, in part, to address some of the environmental concerns regarding potential impacts of dredge disposal operations. Although sediment toxicity was not examined directly, the physical impacts on biota (USACE 1975a) and the bioaccumulation of contaminants from dredged material were evaluated in laboratory and field studies (USACE 1975a,b). Those studies demonstrated the following:

a. Estuarine animals can survive suspended sediment loads in excess of those normally encountered during dredging and disposal.

b. In laboratory exposures to San Francisco Bay sediments, estuarine animals can bioaccumulate trace contaminants.

c. In field studies, contaminant tissue concentrations in animals near the disposal operations were not different from those far removed. The one exception was slightly elevated p,p'-DDE concentrations in mussels, *Mytilus edulis*, during disposal. These differences were not detected 1 month postdisposal.

In 1972, the California Regional Water Quality Control Board (RWQCB) adopted the Jensen criteria (Bowden 1977). These numerical criteria were developed by the U.S. Environmental Protection Agency (USEPA) for freshwater sediment in the Great Lakes and classified sediment as highly polluted, moderately polluted, or slightly polluted, based on bulk sediment chemistry. As research on dredged material progressed, it became clear that these and other chemically based numerical criteria were technically inadequate because they did not assess either bioaccumulation potential or toxicity.

The San Francisco District adopted the use of bioassays for evaluating dredged material in 1980. Regulatory procedures were outlined in Public Notice (PN) 78-1. Elutriate procedures were emphasized since disposal sites in San Francisco Bay were generally dispersive. PN 78-1 also reduced the number of disposal sites from five to three. These were located in the Carquinez Strait, San Pablo Bay, and near Alcatraz Island. To facilitate net export out of the Bay, most dredged material was taken to the Alcatraz disposal site.

In 1982, shoaling was noted at the Alcatraz site. As a result of this important development, the San Francisco District took several steps. The District instituted a slurry policy to enhance dispersion during disposal. They greatly reduced the amount of new dredged material taken to the Alcatraz site and even removed 30 tons (27,200 kg) of construction debris from the site. They monitored the physical configuration of the mound at Alcatraz and found it to be stable after two winter seasons. All of these actions lead to the conclusion that the Alcatraz site could not be considered fully dispersive. Since the majority of dredged material in San Francisco Bay was taken to Alcatraz, a reduction in the capacity of that site represented a major impediment to maintenance dredging and to anticipated new work activities. The San Francisco District formed the Disposal Management Program (DMP) in 1985 and charged it with finding solutions to the disposal problem.

The Long-Term Management Strategy (LTMS) was initiated in 1989 to address increasing environmental concerns and to reflect the San Francisco District's commitment to a LTMS for dredged material. In 1991, the Ocean Disposal Implementation Manual was revised to reflect 14 years of regulatory experience and the many scientific advances that had occurred since 1977 (USEPA/USACE 1991).

Overview of Sediment Toxicity Test Development in the United States

As indicated in the foregoing discussion, the regulation of dredged material disposal in San Francisco Bay has taken advantage of scientific advancements that have occurred elsewhere in the United States. To address concerns specific to the potential toxicity of San Francisco Bay sediments, it is important to have some general knowledge of advances in the field of sediment ecotoxicology. The following is not intended to be a comprehensive review

per se; rather, it is meant to provide the reader with an overview of the advances that have occurred over the past 20 years.

The first peer-reviewed journal article that reported assessment of sediment toxicity was published in 1971 by Gannon and Beeton (1971) (Table 2). The laboratory procedure involved exposing amphipods to freshwater dredged material that had been placed in modified milk cartons. In 1973, recognizing the need for a strong technical base in its regulatory program, the USACE initiated the Dredged Material Research Program (DMRP). Included in the scope of this large program was the development of elutriate and solid phase bioassays to assess potential water column and benthic impacts, respectively (Saucier, Calhoun, and Engler 1978). The bioassays developed during the DMRP were subsequently incorporated into both the Ocean Disposal Implementation Manual (USEPA/USACE 1977) and the interim guidance manual for discharge of dredged or fill material into navigable waters (i.e., the 404 manual) (USACE 1976). These sediment bioassays represented a balance between the state of the art and what could be routinely conducted in a regulatory program.

Prior to the mid-1970s, the scientific community expressed relatively little interest in sediment toxicity. Most of their energies were focused on the fate and effects of environmental contaminants dissolved in aqueous solutions. After the Priority Pollutant List was published in 1976, that emphasis shifted for two reasons. First, it was discovered that many chemicals on the Priority Pollutant List were not very soluble in water and, hence, were not bioavailable. Second, as more field data were gathered, it became apparent that concentrations of many contaminants on the Priority Pollutant List were much higher in the sediment than in the overlying water. Those findings led to initial speculation that sediments might be extremely toxic. However, subsequent research showed that the same forces causing chemicals to partition into the sediments also restricted their bioavailability to aquatic organisms.

A major milestone marking these scientific advances was the 6th Pellston Conference held in 1984 (Dickson, Maki, and Brungs 1984). This was the first time leaders in the scientific community formally met to discuss the fate and effects of sediment-associated contaminants. Bioassay procedures contained in the 1977 USEPA/USACE Ocean Disposal Implementation Manual formed the basis for initial discussion. The researchers reached consensus regarding sediment toxicity (Anderson et al. 1984). They recognized that species sensitivity was related, in part, to the degree of contact between sediment and organism. They recommended amphipods and mysid shrimp for lethal tests and polychaetes, bivalves, oligochaetes, and fish for behavioral or sublethal tests. There was also a strong endorsement of the Tiered Testing Approach for evaluating contaminated sediments (USEPA/USACE 1991). This approach eliminates unnecessary testing and directs limited resources to solving more urgent problems.

Another important milestone in the evolution of sediment toxicity methods occurred in 1987. Members of the American Society for Testing and Materials

(ASTM) created a new Subcommittee, E47.01 Sediment Toxicology. This Subcommittee was charged with identifying technically sound procedures for evaluating sediment toxicity and with drafting appropriate standardized guideline documents. Guidelines, which are in various states of preparation, include the following:

a. Solid Phase Toxicity Tests with Freshwater Invertebrates.

b. Solid Phase Toxicity Tests with Marine Amphipods.

c. Solid Phase Toxicity Tests with Marine Polychaetes.

d. Solid Phase Bioaccumulation Tests with Invertebrates.

e. Solid Phase Bioaccumulation Tests with Fish.

f. Guidance for Designing Sediment Toxicity Tests.

g. Guidance for Collection, Storage, Characterization, and Manipulation of Sediment Prior to Toxicity Testing.

When the USEPA/USACE Ocean Disposal Implementation Manual was first published in 1977, it was a balance between the state of the art and what could be achieved in the regulatory testing environment. It was realized at that time that revisions would have to be made to reflect scientific and regulatory advances. The Manual was revised in 1991. Significant improvements to the current Manual as they relate to sediment toxicity evaluations include the following:

a. Formalizing the Tiered Testing Approach.

b. Refinements to the species selection process.

c. Provisions for evaluating chronic sublethal effects.

The assessment of chronic sublethal effects is treated as a Tier IV assessment and would be carried out only if there is a reason to believe chronic impacts may be occurring and if technically sound test protocols are available.

Scope

The objective of this report is to assess the effect of food ration on the chronic sublethal toxicity of San Francisco Bay sediment. This report is not designed to be used in a regulatory decision-making process (i.e., 404 or 103), but rather is intended to provide input to the District's Disposal Management Plan and LTMS for dredged material disposal in the San Francisco Bay area.

In acute toxicity tests, only one end point is measured, percent survival. In contrast, a plethora of end points exist for sublethal tests. These end points may be categorized according to the level of biological organization they represent. In order of increasing complexity, these levels are as follows: molecular, cellular, tissue, organismic (whole animal), population, and community (Figure 1). When a sublethal effect occurs at any level of biological organization, mechanistic explanations may generally be found at lower levels, while ecological consequences are found at higher levels of complexity.

In the aquatic environment, the ultimate focus of environmental protection is the preservation of viable populations of organisms. Forecasting the potential impact at this level of biological complexity is extremely difficult. Bioassessments at lower levels of complexity (molecular-tissue) are simpler, but their ecological relevance is uncertain. For these reasons, a surrogate toxicological bioassay approach is desirable. This approach, which examines whole animal (organismic) responses, represents a propitious balance between response sensitivity in the sublethal end point and ecological relevance of the results

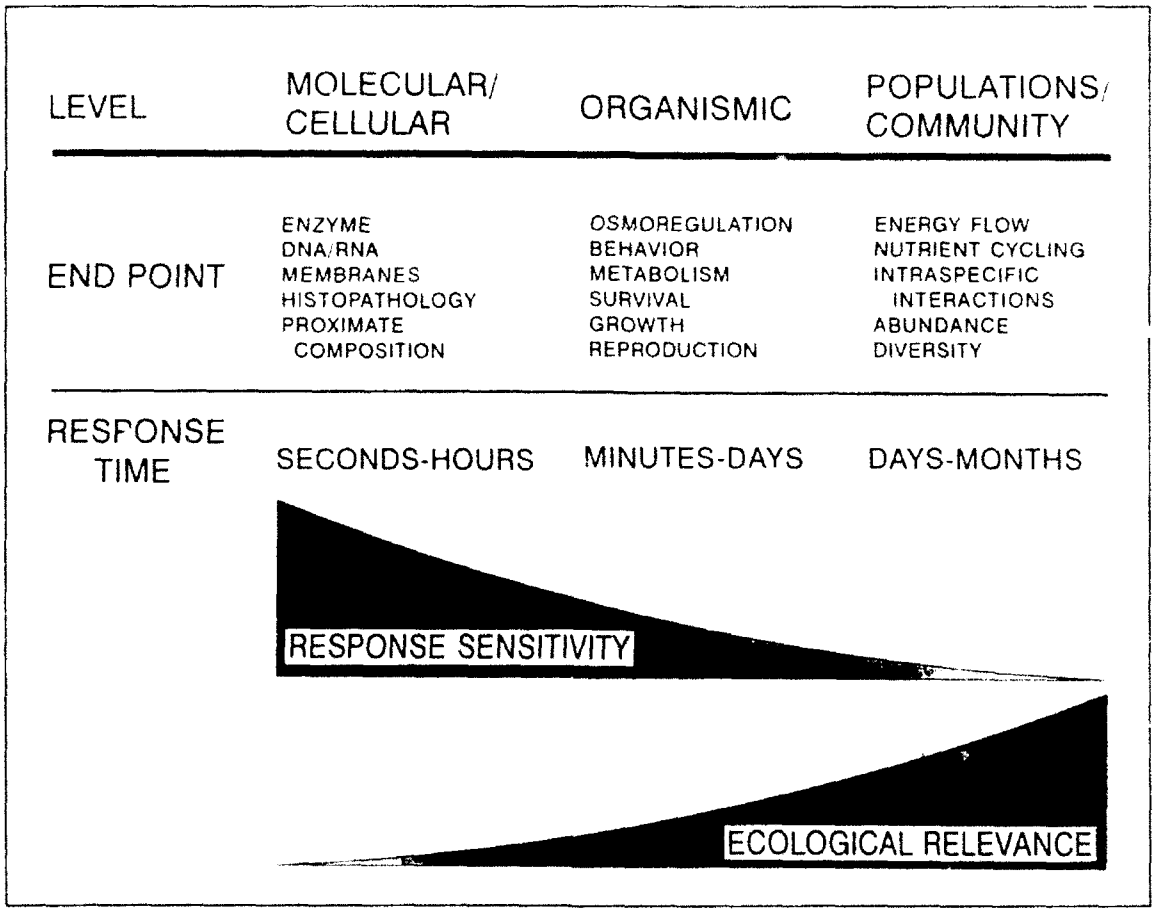

Figure 1. Sublethal end points within levels of biological organization

(Figure 1). Two of the most desirable end points for use in the surrogate toxicological bioassay approach are growth and reproduction. If reproductive success is impaired for a sufficient period of time, the viability of a population may be at risk. In addition, somatic growth and reproductive or gametic growth represent competing energy demands on the bioenergetics of aquatic animals. Therefore, if exposure to contaminated sediment is shown to reduce somatic growth, then reproductive success may also be adversely affected.

Both growth and reproduction are widely accepted end points in the scientific community as ecologically relevant. The California RWQCB, for example, has identified growth as a highly desirable sublethal end point. The board utilizes growth bioassays in its regulatory program for effluent applicants. Test results involving growth and reproduction have the additional benefit of being generally understood and appreciated by a wider nontechnical audience. This latter characteristic is a very important consideration since data for large and/or controversial dredging projects will be carefully scrutinized by the public and, perhaps, the courts.

To have regulatory utility, a sediment bioassay must be able to assess the effects of anthropogenic contaminants in sediment without undue influence from potentially confounding factors. Growth and reproduction are complex processes potentially affected by a host of environmental factors in addition to sediment-associated contaminants. Both growth and reproduction are dependent on the nutritional status of the organism. If nutritionally deprived, the animal may be stressed and thus affected directly or made more susceptible to contaminant effects. Conversely, if there is excess food, water quality may be affected or the animal may feed preferentially on the external food source to the exclusion of the test sediment, thereby, minimizing exposure.

The focus of this report is to evaluate the effect of food ration on the chronic sublethal effects of a contaminated sediment with the marine polychaete *N. arenaceodentata*. A future report will focus on the effect of storage on sediment toxicity.

2 Material and Methods

Test Species

Nereis (Neanthes) arenaceodentata is a benthic infaunal polychaete widely distributed in shallow marine and estuarine benthic habitats of Europe, all three coasts of North America, and Hawaii (Reish 1957, 1963; Sanders et al. 1962; Pettibone 1963; Reish and Alosi 1968; Day 1973; Gardiner 1975; Whitlatch 1977; Taylor 1984). This subsurface deposit-feeder constructs one or more mucoid tubes in the upper 2 to 3 cm of sediment and ingests sediment particles up to 70 µm in diameter with a preference for particles around 12 µm (Whitlatch 1980). *Nereis (Neanthes) arenaceodentata* has been accepted by the regulatory community as an appropriate test species for evaluating sediment (USEPA/USACE 1977, 1991; Johns et al. 1985). A considerable amount of toxicological information on a wide variety of environmental contaminants already exists for this species (Reish 1985; Jenkins and Mason 1988; Anderson et al. 1990).

Taxonomists are still debating the appropriate nomenclature for this species. Pettibone (1963), who suggested *Nereis (Neanthes) arenaceodentata*, lists five other names for this species: *Spio caudatus*, *Nereis (Neanthes) caudata*, *Nereis arenaceodentata*, *Neanthes cricognatha*, and *Neanthes caudata*. Day (1973) dismissed *arenaceodentata* in favor of *acuminata*, which was subsequently used by Gardiner (1975), Taylor (1984), and Weinberg et al. (1990). *Neanthes arenaceodentata* is most commonly used in the toxicological literature. Recent evidence suggests that the North American Atlantic and Pacific populations are genetically dissimilar, reproductively isolated, and probably different species (Weinberg et al. 1990). Until the taxonomic status of this species is resolved, the name most familiar to toxicologists will be used and the original source of worms reported.

The life cycle of *N. arenaceodentata* is well documented, as are culture methods (Reish 1980). As worms approach sexual maturity, males and females establish pairs and occupy a common tube. Eggs are deposited by the female within the tube, and the male presumably fertilizes the eggs at this time. The spent female either exits the tube and dies within 1 to 2 days or is eaten by the male. The male remains in the tube to incubate and guard the

developing eggs. He creates a current of water via rhythmic undulations to remove metabolic waste and prevent hypoxic conditions.

Larval development is direct via nonplanktonic metatrochophore larvae and occurs entirely within the parental tube. Emergent juveniles (EJs) exit the parental tube about 3 weeks after egg deposition. They begin to feed and establish tubes of their own. Juvenile worms grow, and eggs become visible in the coelom of females about 6 weeks postemergence. Egg deposition follows 3 to 7 weeks later. The entire life cycle can be completed in the laboratory in 12 to 16 weeks at 20 to 22 °C. The nonplanktonic benthic larvae and paternal care are unusual among the Nereidae. These features also facilitate laboratory culture and the experimental investigation of sublethal effects on growth and reproduction.

Laboratory Cultures

Stock populations of *Nereis (Neanthes) arenaceodentata* were obtained in March 1988 from Dr. D. J. Reish, California State University at Long Beach. Laboratory cultures were maintained using methods adapted from those described by Reish (1980) and Pesch and Schauer (1988).

Briefly, EJs were raised to sexual maturity in 38-L aquaria containing 30 L of 30-ppt seawater (Instant Ocean) maintained at a temperature of 20 °C. The photoperiod was 12 hr light. Animals were fed a combination of ground Tetramarin flakes (2 mg/worm) and alfalfa (1 mg/worm) twice weekly. This feeding regime was sufficient to maintain adequate water quality in a static-renewal system and has been found to produce survival and reproduction consistent with what has been reported for other laboratory populations of *N. arenaceodentata* (i.e., survival >80 percent; fecundity, ca. 100 to 1,000 eggs/brood; EJ production, ca. 50 to 500 EJs/brood) (Reish 1980; Pesch et al. 1987; Anderson et al. 1990).

Seawater was renewed (80 percent of volume) every 3 weeks. This renewal schedule, based on water-quality monitoring data, was sufficient to maintain good water quality. After 10 weeks, worms were paired using the fighting response (Reish and Alosi 1968) and the presence or absence of eggs in the coelom. Unpaired worms were discarded. Pairs were placed in 600-ml beakers with 500 ml seawater. Gentle aeration was provided via Pasteur pipettes, and the beakers were covered with watch glasses to reduce evaporation. Water was carefully renewed weekly in a manner that avoided disturbing worm pairs.

Beakers were monitored daily for the presence of eggs and EJs. When discovered, EJs were mixed with other broods and returned to the 37-L aquaria to complete the culture cycle. These culture conditions and feeding rations were used in all experiments described below unless otherwise noted.

Test Sediments

Sediments fell into two categories: contaminated sediment (i.e., a San Francisco Bay sediment known to be contaminated) and a control sediment (used in cultures of the test organism). Previous studies evaluating the chronic sublethal toxicity of San Francisco Bay sediments found only minimal effects (Moore and Dillon 1993; Moore and Dillon, "Chronic Sublethal Effects of San Francisco Bay Sediments on *Nereis (Neanthes) arenaceodentata*; Full Life-Cycle Exposure to Bedded Sediments," In Preparation). Selection of the test sediment for this study was based solely on sediment chemistry (i.e., the number and magnitude of contaminants observed) and not toxicity. The contaminated sediment was a composite of several cores taken to project depth (38 ft (11.6 m) below mean low water mark) from areas of Oakland Inner Harbor. Results of chemical analysis indicated this sediment was contaminated with polyaromatic hydrocarbons and metals in the milligram/kilogram range. For purposes of this report, this sediment will be designated as the Oakland Contaminated (OC) sediment. A control sediment from Sequim, WA, was also tested. The Sequim Control (SC) sediment was essentially free of contamination and was tested for comparative purposes. For a detailed discussion of sediment collection, handling, and chemical analysis, see Moore and Dillon (1993).

Experimental Approach

Sediments were evaluated in partial life-cycle exposures with the marine polychaete *Nereis (Neanthes) arenaceodentata*. Sediments were added to 38-L aquaria to a depth of 2.5 cm. Thirty liters of 30-ppt salinity seawater were gently added to each aquarium, carefully avoiding resuspension of the bedded sediment. To initiate the test, emergent juvenile worms (n = 2,000) were taken from laboratory culture and equally distributed among 40 aquaria. Treatments consisted of five food rations (e.g., 2.0X, 1.0X, 0.50X, and 0.25X where X is the normal ration for laboratory cultures) for each of the two sediment types. There were 10 aquaria/treatment (i.e., five replicates for each sediment type; 50 EJs/aquarium). This stocking density has been found to be more than adequate for optimal growth and development of *N. arenaceodentata*. The test was conducted under static-renewal conditions (renewal every 3 weeks) at a temperature of 20 °C and a 12-hr photoperiod. Gentle aeration was provided to each aquaria. Worms were fed twice weekly a combination of finely ground Tetramarin and alfalfa prepared in a seawater slurry. Worms were exposed to test and control sediments for 9 weeks. Dissolved oxygen, salinity, temperature, and pH were monitored weekly. In addition, a 30-ml sample was collected from each aquarium, fixed with 50 µl of 1 *N* HCL, refrigerated, and subsequently analyzed. Total ammonia (milligrams/liter) was determined with an Orion ammonia-specific electrode after adjusting sample pH to 12 with 5 *N* NaOH.

After 9 weeks, worms were removed from all aquaria and counted. Effects on growth were evaluated by measuring the wet weights of surviving animals. Each animal was briefly rinsed in seawater, blotted dry on a lint-free Kimwipe, placed on tared aluminum pans, and weighed to the nearest 0.01 mg on an electrobalance.

Effects on worm reproduction were evaluated by establishing mated pairs (n = 40) from each sediment/food ration treatment and monitoring egg deposition and production of EJs. Sex was confirmed by the presence of eggs in the coelom and the fighting reaction described by Reish and Alosi (1968). Mated pairs were placed in 600-ml beakers covered with watch glasses and provided trickle flow aeration. Animals were fed a Tetramarin-alfalfa slurry to provide enough material for initial foraging and tube-building activity. Pairs were not fed for the remainder of the test since feeding activity is greatly reduced prior to egg deposition and during brood incubation (Pesch and Schauer (1988), personal observation). Approximately 80 percent of the seawater was renewed in each beaker on a weekly basis. Prior to renewal, water quality (dissolved oxygen, salinity, temperature, and pH) was recorded for randomly selected beakers in each treatment group. In addition, a 30-ml sample was collected, fixed with 50 µl of 1 *N* HCL, refrigerated, and subsequently analyzed for total ammonia.

Beakers containing mated pairs were randomly assigned to two types of observations: egg deposition (20 pairs per sediment/food ration treatment) and production of EJs (20 pairs/treatment). All beakers were observed daily for egg masses and EJs. When an egg mass was discovered in beakers assigned to egg deposition, the mass was carefully removed and total number of eggs determined. Beakers assigned to EJ production were terminated when EJs with food in their gut appeared outside the parental tube. EJs were carefully removed and counted. Individual broods were placed on preweighed aluminum pans, dried for 24 hr at 60 °C, and an estimate of individual dry weight obtained by dividing the dry weight by the total number of EJs within a brood. Monitoring for egg deposition and EJ production continued for 10 weeks (Figure 2).

Statistical Analysis

All statistical analysis and data transformation were conducted using SYSTAT statistical software (Wilkinson 1988). All data were screened for homogeneity of variance prior to statistical analysis via Bartlett's test. Effects of each sediment type within a food ration treatment were compared using pooled t-tests. All tests for significance were analyzed at a significance level of $\alpha = 0.05$.

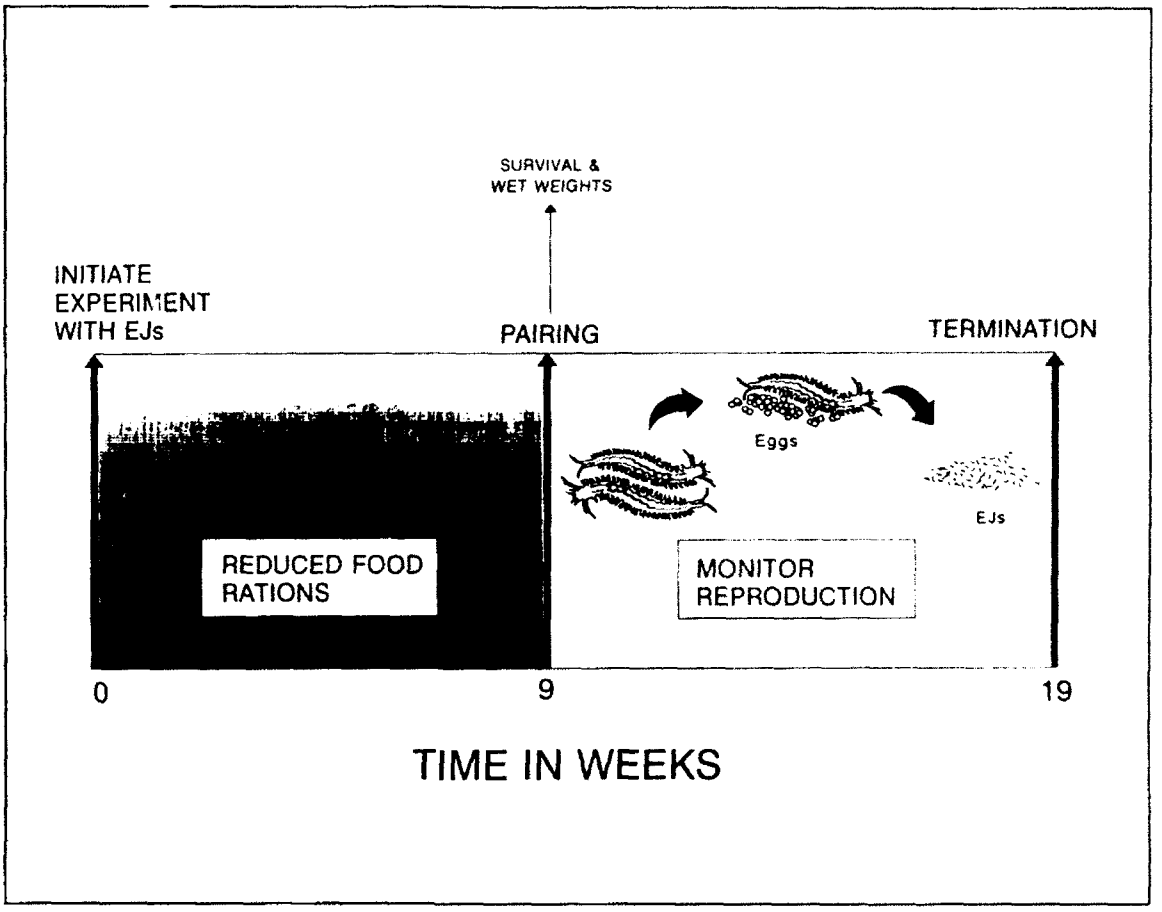

Figure 2. Experimental timetable for exposure of *N. arenaceodentata* to bedded sediments

3 Results

Survival and Growth

Mean worm survival was high (88 to 99 percent) for each of the treatments tested with no statistical difference between the OC and SC sediment treatments (Figure 3, Table 3). Wet weights decreased with decreasing food ration (Figure 4, Table 3). At the 2.0X food ration, there were no significant differences in wet weights between sediment treatments. In the 1.0X and 0.25X treatments, worms in the SC sediments were significantly larger than those reared in the OC sediment (43 versus 36 mg and 13 versuss 9 mg, respectively). While in the 0.50X treatment, worms in the SC sediment were significantly smaller than those in the OC sediment (19 versus 23 mg).

Reproduction

Reproduction was reduced with decreasing food ration. Both egg and EJ production among reproducing pairs was significantly greater in the OC sediment at the 0.50X ration. There were no other statistically significant differences in reproduction between sediment types (Figures 5 and 6, Table 3). In addition to egg and EJ production, two measures of reproductive success were calculated, percent deposition success and percent hatch. Percent deposition success was defined as the percentage of all reproductive pairs/sediment type successfully depositing all eggs within the parental tube. Values for percent deposition success ranged from 35 percent in OC sediment at the lowest (0.25X) ration to 100 percent in OC sediment at the highest (2.0X) ration. Percent deposition success in the OC sediment was higher than control at food rations $\geq$0.50X (Table 3). At the 0.25X ration, percent deposition success was higher in the control. No statistical analysis was performed, since these values were simple point estimates with no associated variance. The experimental design does not allow one to directly determine the proportion of eggs that successfully develop into EJs. However, this parameter can be estimated by dividing the mean number of EJs produced per reproducing pair in each treatment by the corresponding mean fecundity. This calculation yields an estimate of percent hatch (Table 3). Percent hatching success ranged from 60

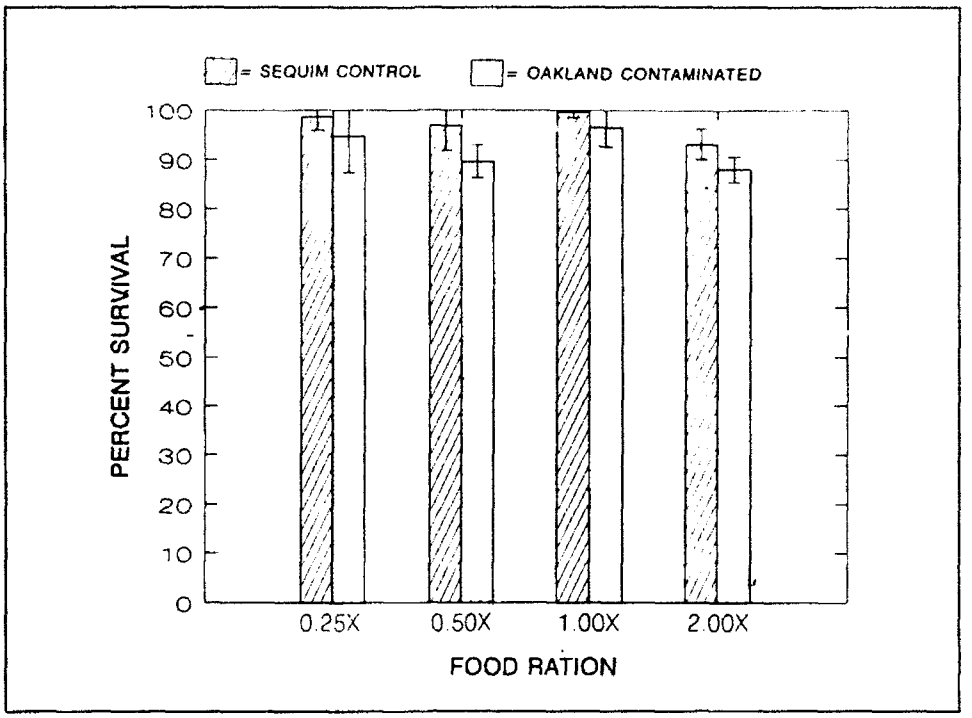

Figure 3. Effect of food ration on mean survival in *N. arenaceodentata* exposed to Oakland Contaminated and Sequim Control sediment. Error bars = standard error of the mean (N = 5)

to 100 percent with no apparent relationship to either food ration or contaminant level. Estimated individual EJ weights were also variable ranging from 14 to 33 µg dry weight with no statistical differences between OC and SC sediment exposures.

Water Quality

Water quality was acceptable in all food ration/sediment treatments (Appendix A).

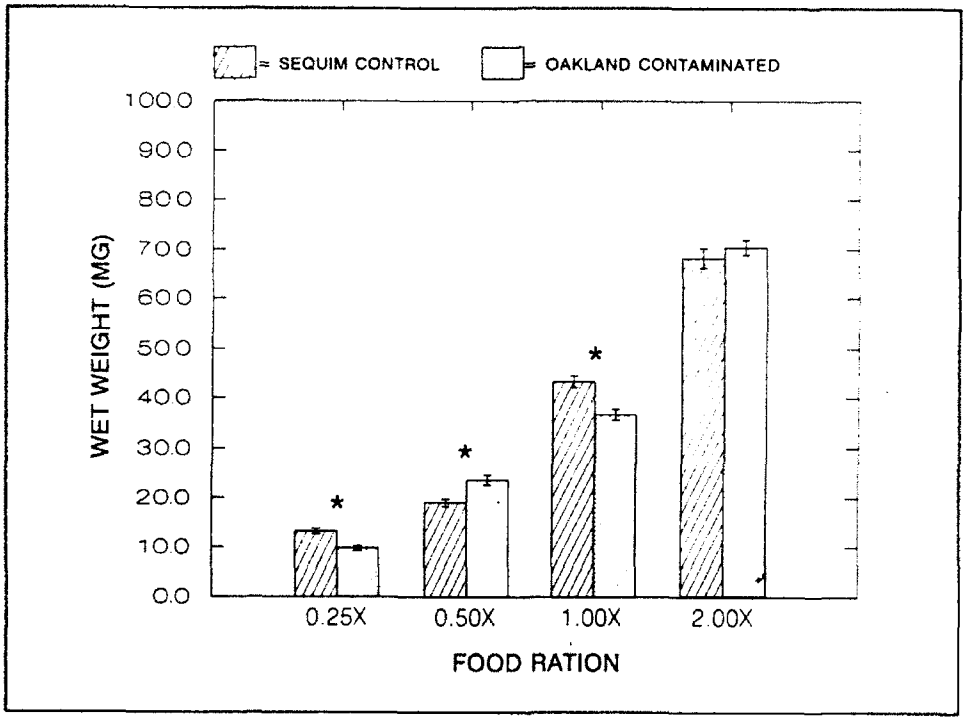

Figure 4. Effect of food ration on mean wet weight (in milligrams) of *N. arenaceodentata* exposed to Oakland Contaminated and Sequim Control sediment. Error bars = standard error of the mean (N = 250). Asterisks indicate significant difference between sediment types within a food ration ($p < 0.05$)

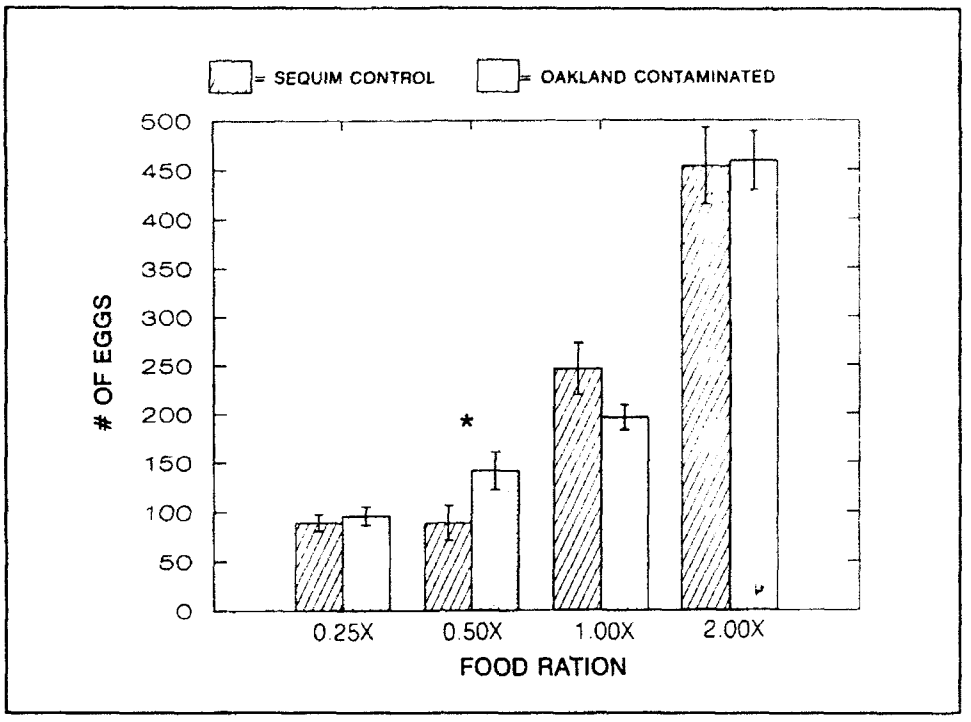

Figure 5. Effect of food ration on mean fecundity (i.e., number of eggs/pair) of *N. arenaceodentata* exposed to Oakland Contaminated and Sequim Control sediment. Error bars = standard error of the mean (N = 20). Asterisk indicates significant difference between sediment types within a food ration ($p < 0.05$)

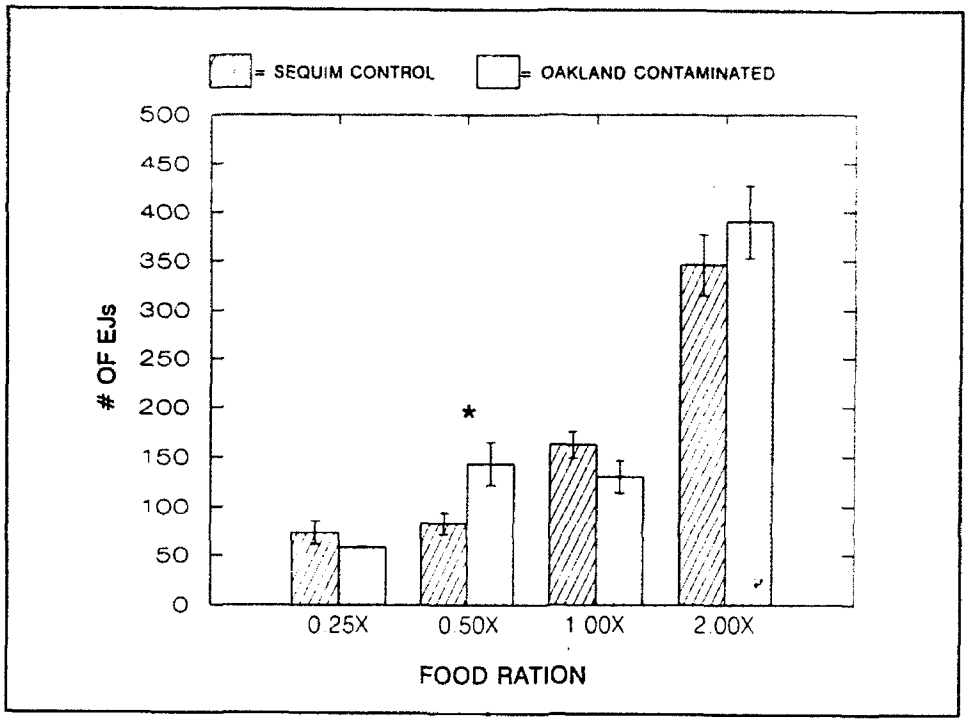

Figure 6. Effect of food ration on mean EJ production of *N. arenaceodentata* exposed to Oakland Contaminated and Sequim Control sediment. Error bars = standard error of the mean (N = 20). Asterisk indicates significant difference between sediment types within a food ration ($p < 0.05$)

4 Discussion

Results of this study demonstrate that sediment bioassay response can be influenced by nutritional factors. Both growth and reproduction decreased with reduced food ration regardless of sediment type. This is not too surprising since the nutritional characteristics of the environment can have important consequences for both growth and reproduction. Organic enrichment of sediments has been shown to be directly related to growth and reproduction in the polychaetes *Capitella* sp. (Tenore 1983; Gremare, Marsh, and Tenore 1989; Tsutsumi et al. 1990), *Streblospio benedicti* (Levin 1986), and *Polydora ligni* (Zajac 1986). Taghon and Greene (1990) showed that as the level of enzymatically available protein in sediments declined, feeding rate increased in the polychaete *Abarenicola pacifica* until a maximum feeding rate was achieved at 0.05 to 0.1 mg protein/gram dry sediment. Above and below this ration, feeding rates declined. Despite this functional adaptation, growth decreased steadily with decreasing protein concentrations.

In *N. arenaceodentata*, egg yolk production is dependent on circulating coelomocytes called eleocytes (Davis and Reish 1975). Eleocytes extract lipids and carbohydrates from the worm's internal musculature and transfer them to developing oocytes. In effect, gametogenesis and subsequent reproductive success are largely dependent on the energy and metabolic substrates assimilated during somatic growth. Consequently, if energy input is reduced, then effects on growth and reproduction would be related as is shown here and in a previous study with *N. arenaceodentata* (Moore and Dillon, "Chronic Sublethal Effects of San Francisco Bay Sediments on *Nereis (Neanthes) arenaceodentata*; Interpretative Guidance for the 21-Day Growth Bioassay," In Preparation).

Both growth and reproduction are integral processes reliant on many biochemical and behavioral factors. In this study, there was no simple correlation between toxicity and sediment contamination (as defined by bulk sediment chemistry). At the 0.50X ration, animals exposed to the OC sediment had higher growth and reproduction, while in the 1.0X and 0.25X rations, controls fared better.

This anomalous response is indicative of the complexity of the processes affecting growth and reproduction and suggests that a combination of mechanisms may be operating to affect these end points. Additional research should

prove beneficial to our understanding of the mechanisms involved and our ability to control the effects of food ration during sediment bioassays.

Evidence from this study does suggests that higher food rations may mask contaminant effects. There were no significant differences in worm survival, growth, or reproduction between sediment exposures at the 2.0X ration. It is speculated that the animals in treatments at the 2.0X ration were able to subsist entirely on the external food source to the exclusion of the test sediment. This speculation is supported by the observation of excess (uneaten) food in treatments at the 2.0X ration. Consequently, animals may not have been ingesting contaminated sediment, and exposure would have been minimized.

It is clear from the results of this and previous studies (Moore and Dillon, "Chronic Sublethal Effects of San Francisco Bay Sediments on *Nereis (Neanthes) arenaceodentata*; Interpretative Guidance for the 21-Day Growth Bioassay," In Preparation) that both growth and reproduction of *N. arenaceodentata* can be manipulated through food ration. This has broad implications for chronic sublethal tests since other species are potentially subject to similar nutritional factors. Consequently, an important component of test development is understanding how food ration influences test end point response. By manipulating food ration to optimize growth and reproduction, robust, highly fecund test organisms that underestimate sediment toxicity may be being created. Conversely, if test conditions do not provide adequate nutrition, the test animal may be stressed and respond to factors other than sediment associated contaminants. While it is impossible for a laboratory bioassay to duplicate field conditions, an attempt to verify the ecological relevance of test conditions (including food ration) should be made. One approach would be to estimate growth rates and fecundity of field populations and use this as a benchmark for evaluating laboratory culture/test methods. Ultimately, ecological relevance should be established through a series of field validation studies. Finally, potential temporal changes in test sensitivity should be monitored through the use reference toxicants and control charts.

5 Conclusions

The effect of food ration on sediment toxicity was evaluated in a chronic partial life-cycle test with the polychaete worm *Nereis (Neanthes) arenaceodentata* exposed to a contaminated San Francisco Bay sediment. Test end points were survival, growth, and reproductive success. Conclusions are summarized below.

- Mean percent survival of *N. arenaceodentata* was high for all food ration/sediment treatments tested.
- Wet weights of *N. arenaceodentata* decreased with decreasing food ration.
- Wet weights in the contaminated and control sediment exposures were significantly different at the three lowest food rations (i.e., 1.0X, 0.50X, and 0.25X). These differences did not correspond to contaminant levels.
- Reproduction (i.e., egg and emergent juvenile production) decreased with decreasing food ration.
- Reproduction (i.e., egg and emergent juvenile production) was significantly higher in the contaminated sediment at the 0.50X ration. No other significant differences in reproduction were observed.
- At the 2.0X ration, there were no significant differences between sediment treatments.

6 Recommendations

Based on the results of this study, the following recommendations are made:

- The effect of food ration on sediment toxicity should be evaluated with a sediment shown to induce a chronic sublethal response in *N. arenaceodentata.*

- Fecundity in natural populations of *N. arenaceodentata* should be determined to provide a basis for food ration and corresponding reproduction in laboratory populations.

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Table 1
Milestones in the Regulation of Dredged Material in San Francisco Bay

1965	Committee on Tidal Hydraulics suggests San Francisco District (CESPN) may be redredging a significant amount of material.
1970	Passage of the National Environmental Policy Act.
1970	CESPN initiates Dredge Disposal Study. Terminated in 1975.
1972	CESPN reduces the number of in-bay disposal sites from 11 to 5.
1972	California RWQCB adopts USEPA's Jensen bulk sediment criteria. Material classified as "polluted" by these criteria was either placed upland or taken offshore to the 180-m ocean disposal site.
1973	USACE initiates Dredged Material Research Program.
1976	USACE publishes interim guidance manual for implementation of section 404 (b) of Public Law 92-500.
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual.
1978	Public Notice 78-1 (PN 78-1) was drafted by the CESPN. Elutriate test procedures adopted from the Ocean Disposal Implementation Manual, and in-bay disposal limited to three dispersive sites (Alcatraz, San Pablo Bay, and Carquinez Strait)
1980	California RWQCB adopts PN 78-1.
1980	100-fathom ocean disposal site becomes part of the Point Reyes-Farallon Islands Marine Sanctuary and is subsequently removed from the final designation process by USEPA.
1982	Mounding at the Alcatraz site noted in November.
1984	CESPN implements slurry policy to enchance dispersion during disposal.
1985	CESPN establishes the Disposal Management Program to find operational, environmentally acceptable solutions to disposal problems.
1985	San Francisco Bar Channel ocean disposal site receives final designation by USEPA. It can receive only coarse-grained material.
1988	Bioassay procedures used to evaluate Oakland Inner Harbor sediments under section 401 of the Clean Water Act.
1989	The Long-Term Management Strategy was initiated to reflect increasing regulatory and environmental concerns related to dredged material disposal in San Francisco Bay.
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

Table 2
Milestones in the Scientific Development of Sediment Toxicity Tests

1971	Gannon and Beeton publish first journal article on sediment bioassays
1973	USACE initiates Dredged Material Research Program (DMRP).
1976	Publication of Priority Pollutant List by USEPA.
1976	Publication of USACE 404 manual.
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual
1978	DMRP completed
1984	Pellston Conference on Fate and Effect of Sediment-Bound Chemicals
1987	Formation of ASTM Subcommittee E47.03 on Sediment Toxicology
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

Table 3
Effect of Food Ration on Survival, Growth (mg wet weight), and Reproduction of *N. arenaceodentata* Exposed to Oakland Contaminated (OC) and Sequim Control (SC) Sediment

Food Ration	2.0X		1.0X		0.5X		0.25X	
Sediment Sample	SC	OC	SC	OC	SC	OC	SC	OC
Life History Trait								
Survival, %	93 (3)	88 (3)	99 (1)	96 (4)	96 (5)	90 (3)	98 (2)	95 (7)
Wet weight	68.0 (1.8)	70.2 (1.5)	42.9 (1.0)	36.2* (0.8)	18.6 (0.5)	23.2* (0.8)	12.8 (0.4)	9.4* (0.3)
Egg Production								
All pairs	430 (43)	459 (30)	207 (31)	186 (16)	44 (13)	106* (20)	54 (11)	34 (11)
Reproducing pairs only	453 (39)	459 (30)	245 (27)	196 (13)	89 (17)	141* (19)	90 (8)	96 (9)
EJ Production								
All pairs	311 (36)	351 (43)	130 (16)	98 (18)	58 (12)	86 (20)	33 (10)	6* (4)
Reproducing pairs only	346 (31)	390 (37)	162 (13)	130 (16)	83 (11)	144* (21)	73 (12)	58 (1)
Deposition success,[1] %	95	100	79	90	45	70	60	35
Hatching success,[2] %	76	84	65	66	93	100	81	60
EJ size[3]	21 (2)	23 (2)	33 (5)	25 (2)	28 (3)	29 (5)	28 (4)	14 (8)

Note: Asterisk indicates significant difference between sediment types within a food ration ($p < 0.05$). EJ = Emergent Juvenile worms.

[1] # pairs successfully depositing all eggs inside the tube/# pairs.

[2] Est. from (mean EJs/reproducting pair)/(mean eggs/reproducing pair).

[3] Est. for each brood from total EJ biomass (µg dry)/# EJs.

Appendix A
Water Quality Parameter Monitoring

WATER QUALITY

WATER QUALITY PARAMETERS DURING 70 EXPOSURE TO BEDDED SEDIMENTS, MEAN (SE) (N = 60)

SEDIMENT SAMPLE	TREATMENT	TEMP. (°C)	SAL. (ppt)	D.O. (mg/L)	pH	TOTAL NH_3 (mg/L)
SC	0.25X	20.5 (0.05)	30.0 (0.07)	7.01 (0.056)	7.95 (0.027)	0.04 (0.011)
SC	0.50X	20.5 (0.06)	30.0 (0.11)	6.86 (0.060)	7.94 (0.024)	0.08 (0.027)
SC	1.00X	20.5 (0.05)	30.0 (0.08)	6.85 (0.054)(	7.87 0.024)	0.07 (0.014)
SC	2.00X	20.4 (0.05)	29.9 (0.08)	6.66 (0.072)	7.82 (0.030)	0.12 (0.021)
OC	0.25X	20.5 (0.05)	30.0 (0.06)	7.03 (0.045)	7.91 (0.024)	0.02 (0.013)
OC	0.50X	20.5 (0.05)	30.0 (0.07)	6.89 (0.055)	7.88 (0.020)	0.04 (0.009)
OC	1.00X	20.4 (0.05)	30.0 (0.07)	6.86 (0.055)	7.82 (0.021)	0.07 (0.013)
OC	2.00X	20.4 (0.05)	30.0 (0.07)	6.71 (0.068)	7.72 (0.025)	0.13 (0.027)

WATER QUALITY CONTINUED

WATER QUALITY PARAMETERS DURING REPRODUCTIVE MONITORING, MEAN (SE) (N = 55)

SEDIMENT SAMPLE	TREATMENT	TEMP. (°C)	SAL. (ppt)	D.O. (mg/L)	pH	TOTAL NH_3 (mg/L)
SC	0.25X	20.2 (0.07)	30.1 (0.07)	7.28 (0.069)	7.95 (0.014)	0.13 (0.029)
SC	0.50X	20.2 (0.07)	30.1 (0.07)	7.32 (0.065)	7.97 (0.011)	0.10 (0.033)
SC	1.00X	20.2 (0.08)	30.0 (0.06)	7.36 (0.076)	7.92 (0.012)	0.27 (0.064)
SC	2.00X	20.2 (0.08)	29.9 (0.07)	7.37 (0.099)	7.92 (0.016)	0.37 (0.089)
OC	0.25X	20.2 (0.06)	30.1 (0.07)	7.93 (0.014)	7.94 (0.014)	0.09 (0.026)
OC	0.50X	20.2 (0.07)	30.0 (0.07)	7.37 (0.081)	7.98 (0.017)	0.21 (0.064)
OC	1.00X	20.2 (0.08)	30.0 (0.07)	7.31 (0.092)	7.95 (0.013)	0.34 (0.073)
OC	2.00X	20.2 (0.08)	30.0 (0.07)	7.44 (0.07)	7.93 (0.010)	0.43 (0.079)

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13. ABSTRACT *(Maximum 200 words)*

This report is designed to address concerns regarding the effect of food ration on toxicity during chronic sublethal sediment bioassays. To this end, a contaminated San Francisco Bay sediment and a "clean" control sediment were evaluated in a chronic sublethal test under a series of different food rations, with the marine polychaete worm *Nereis (Neanthes) arenaceodentata*. Animals were exposed from early juvenile stage through the onset of gametogenesis. Treatments were 2.0X, 1.0X, 0.5X, and 0.25X where X is the recommended food ration for laboratory cultures. Test end points were survival, growth, and reproduction.

The contaminated sediment was a composite of several cores taken to project depth (38 ft (11.6 m) below mean low water mark) from an area in Oakland Inner Harbor known to be contaminated with polycyclic aromatic hydrocarbons and metals. Comparisons were made with a clean control sediment. The control sediment is used in the laboratory cultures of *N. arenaceodentata* and was collected from Sequim, WA.

Mean percent survival of *Neanthes* was high (>90 percent) in both the contaminated and control sediment across all food ration treatments. Individual wet weights were significantly reduced with decreasing food ration in both contaminated and control sediments. Significant differences in wet weight between sediment types were observed at the 1.0X, 0.5X, and 0.25X rations. Reproduction (fecundity and emergent juvenile (EJ) production) was also

(Continued)

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Chronic sublethal	*Neanthes*	37
Dredged material	San Francisco Bay	16. PRICE CODE
Food ration	Sediment	

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